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Effects of Dieldrin (HEOD)[1] and Some of its Metabolites on Synaptic Transmission in the Frog Motor End-Plate

L. M. A. AKKERMANS, J. VAN DEN BERCKEN, J. M. VAN DER ZALM AND H. W. M. VAN STRAATEN

Institute of Veterinary Pharmacology and Toxicology, University of Utrecht, Biltstraat 172, Utrecht, The Netherlands

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The effects of HEOD and some of its metabolites on synaptic transmission in the frog motor end-plate were studied by means of intracellular microelectrodes. HEOD itself and the metabolites 9-*syn*-hydroxy-HEOD and the aldrin-derived dicarboxilic acid had no significant effect on frequency and amplitude of miniature end-plate potentials, nor on end-plate membrane potential. In sharp contrast with this aldrin-transdiol (6,7-*trans*-dihydroxy-dihydro-aldrin) was very potent in exerting both pre- and postsynaptic actions. This metabolite caused a rapid and marked increase in miniature end-plate potential frequency, together with a decrease in their amplitude. Evidence is presented suggesting that the spontaneous transmitter release is enhanced by two prejunctional mechanisms: partly by a calcium-dependent effect, probably a depolarization of the nerve terminal, and partly by a calcium-independent action. Another typical prejunctional action of aldrin-transdiol is the reduction of the amount of transmitter released in response to high external potassium concentration. Aldrin-transdiol also affected the evoked transmitter release and caused a marked increase in end-plate potential amplitude followed by a decrease, and finally a complete blockade of neuromuscular transmission was observed. This transient increase in transmitter release was shown to be due to a transient increase in quantal content. The subsequent fall in end-plate potential amplitude and the fall in miniature end-plate potential amplitude are probably the result of a reduction of the sensitivity of the postsynaptic membrane to acetylcholine as demonstrated by ionophoretic application of this transmitter. There was no aldrin-transdiol-effect on the end-plate membrane potential. The present results strongly support the hypothesis that HEOD must be converted to aldrin-transdiol before it can exert its neurotoxic action.

INTRODUCTION

Although the ability of dieldrin (HEOD) and other cyclodiene insecticides to alter nervous system function has been extensively investigated, not much is known about their precise site and mode of action. Unlike DDT, dieldrin, even in high concentrations, has no significant action on axonal nerve membrane (1–3). The skeletal muscle can also be excluded as a primary site of action (4, 5). The effect of dieldrin on sense organs still represents a puzzle. In the lateral-line sense organ of *Xenopus* no dieldrin-effect was observed (6, 7). In the cockroach, Giannotti *et al.* (1) observed an absence of dieldrin symptoms peripheral to the central ganglia. Wang *et al.* (8), on the other hand, observed that dieldrin produced repetitive trains of impulses in the cockroach leg, which were much less intense than those caused by DDT. The latter authors stated that apparently the

[1] Dieldrin contains 85% w/w of HEOD.

sensory neurons play a minor role in the development of the dieldrin poisoning symptoms.

Evidence has been produced that dieldrin, albeit after a long latency, interferes with the synaptic transmission in the central nervous system of the cockroach, causing an increase in spontaneous activity and a prolonged postsynaptic after-discharge in response to a single presynaptic stimulus (1, 8–10). According to Shankland and Schroeder (10) these effects may be due to an excessive and spontaneous release of presynaptic stores from cholinergic nerve terminals.

Wang *et al.* (8) found that aldrin-transdiol(6,7-*trans*-dihydroxydihydroaldrin), one of the metabolites of dieldrin, affected the metathoracic ganglion much more rapidly and potently than dieldrin itself and all other dieldrin derivatives tested. These authors concluded that it is reasonable to assume that aldrin-transdiol is one of the active forms of dieldrin in the cockroach. Since aldrin-transdiol is also one of the major metabolites of dieldrin in several vertebrates (11–14) it is important to study the effects of this compound on vertebrate synaptic transmission.

In the present study the effects of HEOD, aldrin-transdiol and two other metabolites, the aldrin-derived dicarboxilic acid (14) and 9-*syn*-hydroxy-HEOD (15), on the frog motor end-plate were investigated further to define the mechanisms of action of these compounds on synaptic transmission. It is beyond question that these effects on neuromuscular synaptic transmission, as they are described here, could direct attention to the generally believed effects of cyclodiene insecticides on the mammalian central nervous system (16–19). A preliminary report of some of this material has appeared (20).

MATERIALS AND METHODS

Experiments were performed on sartorius and extensor longus digiti IV nerve–muscle preparations isolated from pre-chilled, decapitated male and female frogs (*Rana esculenta*). In a few experiments *Rana temporaria* or *Rana pipiens* was used. The muscle was mounted with its medial side upward in a Perspex bath and stretched to 1.2 times its maximal length *in situ*.

End-plate potentials (e.p.p.'s), intracellular and focal extracellular spontaneous miniature end-plate potentials (m.e.p.p.'s), acetylcholine potentials (ACh-potentials) and end-plate membrane potentials were recorded with glass microelectrodes, filled with 3 *M* KCl, having resistances of 8—15 Mohm and tippotentials less than 5 mV. During the experiments the microelectrode was held in the same end-plate region of surface muscle fibres for periods as long as 5 hr, without an appreciable effect on the end-plate membrane potential. Non-polarizable Ag–AgCl electrodes were used to earth the Ringer's solution in the bath and to connect the microelectrode to an electrometer amplifier with input capacitance neutralization.

The muscle was continuously superfused with Ringer's solution containing (in m*M*): NaCl 115, KCl 2.5, Na_2HPO_4 2.15, NaH_2PO_4 0.85 and $CaCl_2$ 2.0; pH 7.1–7.3. The rate of perfusion was 6 ml per min and in about 5 min 95% of the bathing solution was changed. The composition of the Ringer's solution was altered in several experiments by isotonic substitution for sodiumchloride. HEOD (99.4% 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*endo*-1,4-*exo*-5,8-dimethanonaphtalene), aldrin-transdiol (ATD) (1,2,3,4,10,10-hexachloro-6,7-*trans*-dihydroxy-1,4-*endo*-5,8-*exo*-dimethano-1,4,4a,5,6,7,8,8a-octahydronaphtalene) and 9-*syn*-hydroxy-HEOD (9HH) (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-9-hydroxy-*endo*-1,4-*exo*-5,8-dimethanonaphtalene) were dissolved in 96% ethanol to make up stock solutions. This was squirted forcefully through a No. 27 hypodermic needle into Ringer's solution to give a fine suspension. The final concentration of ethanol was respectively in the experiments

with HEOD less than 0.4% and with ATD and 9HH less than 0.1%. Additional experiments were done to study the possible effects of ethanol. The aldrin-derived dicarboxylic acid (ADA) (4,5,6,7,8,8a-hexachloro-1,2,3a,4,7,7a-hexahydro-*exo*-4,7-methanoindene-1,3-dicarboxylic acid) was dissolved in distilled water with Na_2HPO_4 at pH 8 to make a stock solution.

The temperature in the bath was kept at 18 ± 0.2°C with the aid of a thermoelectric device. Temperature and end-plate membrane potential were continuously recorded on strip chart recorder. On the same recorder m.e.p.p. frequency was continuously monitored by means of an analogue frequency-registering system; time constant 2.5 sec. Additional recordings were made on magnetic tape for further analysis of the amplitude and frequency of occurrence of m.e.p.p.'s on computer. Only recordings with a clear separation between m.e.p.p.'s and base line noise were analysed. At lower rates (<1 sec^{-1}) the mean frequency was determined over successive periods of 100 sec and at higher rates (>1 sec^{-1}) over successive periods of 10 sec. E.p.p.'s were recorded in Ringer's solution with a low calcium–magnesium ratio (0.4 m*M* Ca^{2+}, 3 m*M* Mg^{2+}) and photographed from oscilloscope traces or automatically averaged.

A suction electrode was used to stimulate the motor nerve supramaximally with rectangular pulses of 0.1 msec, delivered through a photoelectric isolation unit, at frequencies of 0.5–2.0 per sec. The mean number of packets of transmitter released by a nerve impulse, i.e., quantal content (m), was determined in two ways: m = (mean e.p.p. size)/(mean m.e.p.p. size) or m = ln (total number of impulses/number of e.p.p. failures) (21). The results of the two methods were approximately congruous. In order to obtain a statistically reliable measure of m 256 e.p.p.'s were averaged (22). When m exceeded 10, a correction factor for non-linear summation was used (23).

Ionophoretic application of acetylcholine (ACh) was used to measure the sensitivity of the postjunctional membrane to this transmitter (24, 25). The outward current pulse which was delivered by a modulated current source had an intensity of 10^{-6}–10^{-7} A and a duration of 5–200 msec. Both intensity and duration were kept constant during an experiment. An inward current was used to prevent the diffusion of ACh from the ACh-filled (2 *M*) pipette. The ACh-potentials were evoked every 2 min throughout the experiment and oscilloscope traces were photographed.

RESULTS

HEOD

Six nerve–muscle preparations were exposed to HEOD in concentrations ranging from 10^{-5}–10^{-4} *M* for more than 3 hr. In none of these experiments an effect of HEOD on m.e.p.p. frequency or amplitude was observed. In experiments with 5×10^{-4} *M* HEOD (0.4% ethanol) there was a slight increase in m.e.p.p. frequency. This could be attributed to ethanol, however, because in control experiments in which the preparation was exposed to Ringer's solution containing 0.4% ethanol only, a similar increase in m.e.p.p. frequency was observed. HEOD did not affect the end-plate membrane potential.

Aldrin Transdiol (ATD)

Effects on m.e.p.p. frequency. The m.e.p.p. frequency of untreated end-plates in normal Ringer's solution ranged from 0.1–5.6 per sec. In all 120 end-plates tested, ATD in concentrations ranging from 10^{-5}–10^{-4} *M* caused a dramatic increase in m.e.p.p. frequency. In most experiments the m.e.p.p. frequency began to increase within a few minutes after the addition of ATD, while in other experiments there was a delay of more than 10 min before an ATD-effect showed itself. Figure 1A shows the effect of 2.5×10^{-5} *M* ATD on m.e.p.p. frequency in 5 different end-plates. It will be

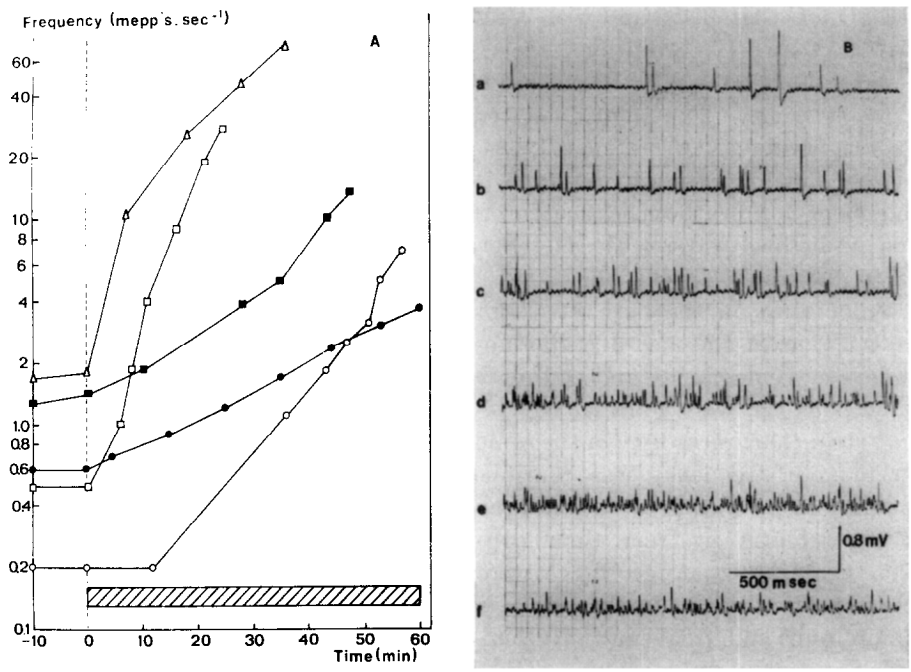

FIG. 1A. *Time course of effect of ATD on m.e.p.p. frequency in five different preparations (semi-logarithmic plot). ATD in a concentration of 2.5 × 10⁻⁵M was added at time 0 and was present throughout the experiments as indicated by the hatched bar. B. Effect of ATD on m.e.p.p. frequency and amplitude. Registrations (ac-coupled) were made before (a) and at 21, 35, 47, 55 and 70 min after application of 2.5 × 10⁻⁵ M ATD respectively (b–f). Note that in f most m.e.p.p.'s are lost in base line noise.*

noticed that the rate of m.e.p.p. frequency increase is different for each end-plate, although the rate of perfusion of the bath remained the same.

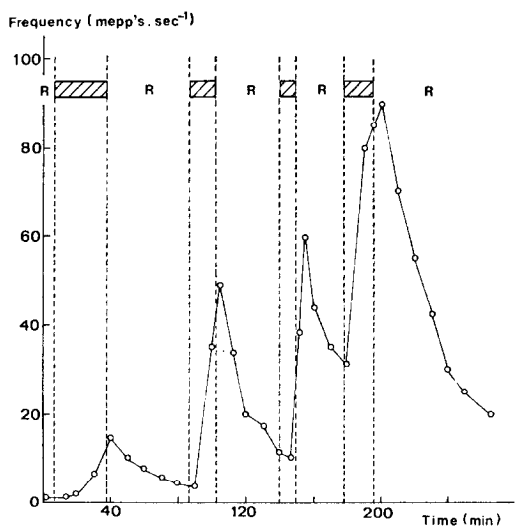

FIG. 2. *Reversibility of the effect on m.e.p.p. frequency of subsequent ATD-applications. Normal Ringer's solution (R) was alternated with Ringer's solution containing 5 × 10⁻⁵ M ATD (hatched bars).*

In addition to this m.e.p.p. frequency accelerating effect ATD also caused a marked suppression of the m.e.p.p. amplitude, as is illustrated in Fig. 1B, and ultimately all m.e.p.p.'s disappeared in the base line noise. Because of this we were not able to see a maximum of the m.e.p.p. frequency. The blocking action of ATD will be described in greater detail in the next paragraph.

Figure 2 shows that by washing the preparation with normal Ringer's solution it was possible to reverse the ATD-effect. Subsequent ATD applications caused a faster and much greater increase in m.e.p.p. frequency, while reversibility became less. It was remarkable throughout that during the washout period spontaneous high frequency bursts of m.e.p.p.'s of about 0.5 sec in duration occurred. In a few

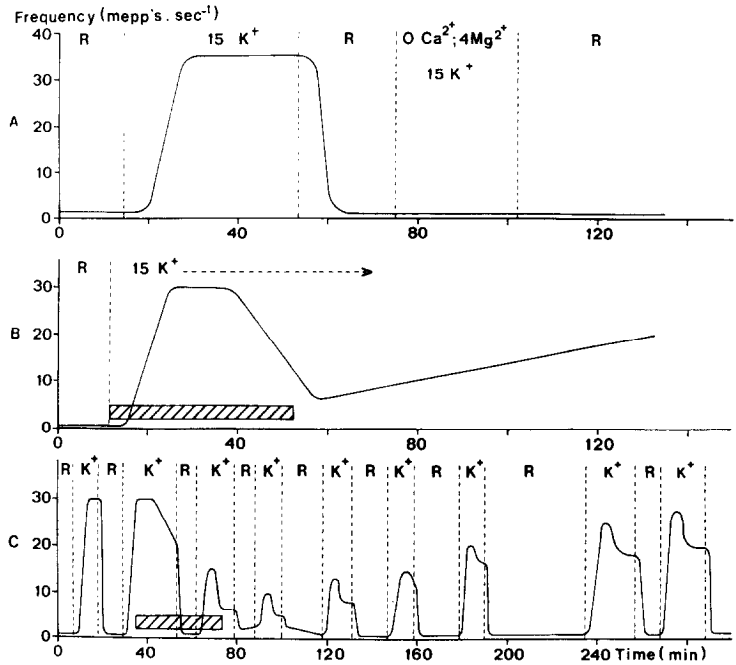

FIG. 3A. *Alterations in m.e.p.p. frequency in an untreated preparation upon switching from normal Ringer's solution (R) to Ringer's solution containing 15 mM K^+ (15 K^+) and back to normal Ringer's solution. Subsequent perfusion with Ringer's solution containing 15 mM K^+, but 4 mM Mg^{2+} and no added Ca^{2+} (0 Ca^{2+}; 4 Mg^{2+}; 15 K^+) does not yield a change in m.e.p.p. frequency. B. Effect of 2.5 × 10^{-5} M ATD (hatched bar) on m.e.p.p. frequency accelerating effect of 15 mM K^+ (15 K^+). C. M.e.p.p. frequency in normal Ringer's solution (R) and in Ringer's solution containing 15 mM K^+ (K^+) before, during and after the application of 2.5 × 10^{-5} M ATD (hatched bar). Note that at about 100 min after the start of the experiment the maximum suppression of the K^+-response coincides with the maximum increase in frequency in normal Ringer's solution. The graphs are tracings of registrations made with the aid of an analogue frequency-registering system with a time constant of 2.5 sec.*

experiments a concentration of 10^{-4} *M* ATD was used. Within 10 min after ATD application the m.e.p.p. frequency rose to more than 60 per sec until the amplitude became so small that reliable frequency measurements could no longer be taken. After these preparations had been washed for more than 2 hr the m.e.p.p.'s did not reappear.

In separate control experiments a possible effect of ethanol on m.e.p.p. frequency was tested. In a concentration of 0.1%, the maximum concentration used in the ATD experiments, ethanol did not cause an increase in m.e.p.p. frequency. Even a concentration of 0.2% ethanol was without effect. These observations agree with Okada's (26).

In order to obtain more insight in the m.e.p.p. frequency accelerating effect of ATD, the interference of this drug with the effects of changing the Ca^{2+}- and K^+-concentrations of the Ringer's solution was studied. Raising the K^+-concentration of the Ringer's solution from 2.5–15 m*M* caused an increase in m.e.p.p. frequency from about 1 per sec to about 35 per sec in untreated preparations (Fig. 3A). This augmented spontaneous transmitter release which could be sustained for more than 2 hr was quickly reversible upon returning to normal Ringer's solution. It is common knowledge that this depolarization coupled m.e.p.p. frequency potentiating effect requires the presence of Ca^{2+} and is inhibited by Mg^{2+} (27, 28). This is

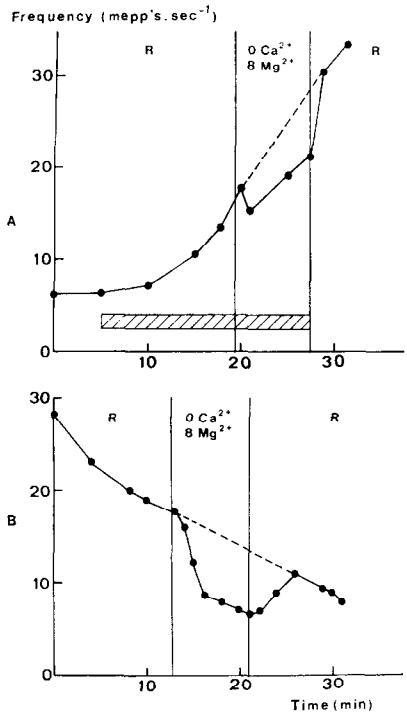

FIG. 4. *Effect of switching from normal Ringer's solution (R) to Ringer's solution containing 8 m*M *Mg^{2+} and no added Ca^{2+} (0 Ca^{2+}; 8 Mg^{2+}) and switching back to normal Ringer's solution on m.e.p.p. frequency during exposure to 2.5×10^{-5} M ATD (hatched bar) (A) and during the washing-out after exposure of the preparation to ATD for 40 min (B). In both figures the expected time course of the m.e.p.p. frequency, had the perfusion with normal Ringer's solution not been interrupted, is indicated by a dashed line.*

illustrated in Fig. 3A which shows that the m.e.p.p. frequency potentiating effect of 15 m*M* K^+ was completely absent in Ringer's solution containing 4 m*M* Mg^{2+} and no added Ca^{2+}. Figure 3B shows that after ATD was added the increase in m.e.p.p. frequency by 15 m*M* K^+ could not be sustained and after about 20 min the m.e.p.p. frequency gradually declined from 30 per sec to 6.5 per sec. Washing the preparation with Ringer's solution containing 15 m*M* K^+ caused a slow increase in m.e.p.p. frequency, but after 60 min the original level had not yet been reached. This indicates that the K^+-antagonistic effect of ATD is longlasting and is difficult to wash out. This was studied further in experiments in which the normal Ringer's solution was alternated with Ringer's solution containing 15 m*M* K^+. A typical example of such an experiment is illustrated by Fig. 3C. When ATD was applied during the perfusion with 15 m*M* K^+–Ringer's solution the m.e.p.p. frequency declined in the same way as in the previous experiments. At a subsequent application of 15 m*M* K^+ the increase in m.e.p.p. frequency was greatly suppressed and in addition the frequency dropped to a lower level within about 5 min. This was even more noticeable during the next period of perfusion with 15 m*M* K^+–Ringer's solution, although ATD had already been withdrawn from the bath. Meanwhile, ATD also caused an increase in m.e.p.p. frequency in normal Ringer's solution which reached a maximum at about 100 min after the start of the experiment. The maximum suppression of the K^+-response always coincided with the maximum increase in m.e.p.p. frequency in normal Ringer's solution. At 120 min after the start of the experiment, when the m.e.p.p. frequency in normal Ringer's solution was almost back to normal, the K^+-response was still markedly inhibited. Even more than 180 min after the withdrawal of ATD the K^+-response is not yet back to normal. These experiments clearly demonstrate that ATD has two distinct effects on the spontaneous transmitter release. It causes an increase in m.e.p.p. frequency in normal Ringer's solution, and it suppresses the m.e.p.p. frequency potentiating effect of an increase in external K^+-concentration.

The m.e.p.p. frequency accelerating effect of ATD developed fully in Ringer's solution with no added Ca^{2+} with or without 2 m*M* EDTA, and there was no obvious difference from the experiments in normal Ringer's solution. Similar experiments with 2 m*M* EGTA (ethyleneglycol bis(aminoethylether)-N,N'-tetra-acetic acid) were not successful, because the mem-

brane potential was not stable and declined slowly to 50 mV or less (29). However, when after ATD application the normal Ringer's solution was replaced by Ringer's solution containing 8 m*M* Mg^{2+} and no added Ca^{2+}, there was a drop in m.e.p.p. frequency as illustrated in Fig. 4A. After that the m.e.p.p. frequency recontinued to increase, although with a somewhat slower time course. A similar drop in m.e.p.p. frequency was observed when during the washout period the fluid in the bath was switched to Ringer's solution with 8 m*M* Mg^{2+} and no added Ca^{2+} (Fig. 4B). In both cases, switching back to normal Ringer's solution caused an increase in m.e.p.p. frequency to a level which would be reached if the perfusion with normal Ringer's solution had not been interrupted. In 4 other experiments a similar drop in m.e.p.p. frequency during perfusion with Ringer's solution containing 8 m*M* Mg^{2+} and no added Ca^{2+} was observed.

Effects on m.e.p.p. and e.p.p. amplitude. In most experiments the m.e.p.p. amplitude began to decrease within several minutes after exposure to ATD and in the end all m.e.p.p.'s disappeared in the base line noise (Fig. 1B). This effect occurred in normal as well as in the modified Ringer's solutions. In three experiments a fall in amplitude of extracellularly focally recorded m.e.p.p.'s was also observed. In this connection it is worth noting that ATD had no effect on the end-plate membrane potential; its value remained constant before and after the application of the drug, i.e., 84 ± 5 mV (mean ±SD, $n = 120$).

ATD also had a marked effect on the evoked transmitter release causing a transient increase in e.p.p. amplitude followed by a gradual suppression. This is illustrated in Fig. 5 which shows averaged e.p.p.'s before and at intervals after exposure of the preparation to 2.5×10^{-5} *M* ATD. The e.p.p. amplitude began to show a marked increase after ATD application and reached a maximum of more than 3 times control value in about 10 min. After that the e.p.p.

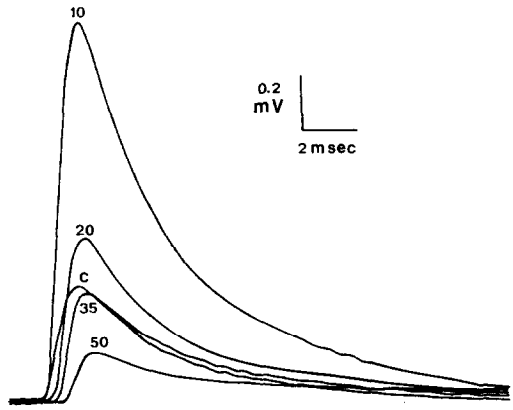

FIG. 5. *Automatically averaged e.p.p.'s* (n = *256*) *before* (*C*) *and at intervals after the addition of* 2.5×10^{-5} M *ATD to the bath. Note the increase in delay between stimulus artifact* (*not shown in the figure*) *and the start of the e.p.p. Numbers indicate time in minutes.*

decreased gradually till ultimately it was completely blocked. Figure 5 also shows that the delay between the stimulus artifact (not shown in the figure) and the e.p.p. increased gradually during the ATD treatment. The time course of the averaged e.p.p.'s was not affected by the drug; time to peak and half time of decay remained unaffected, 1.3 and 2.8 msec respectively ($n = 256$, Fig. 5). Higher concentrations of ATD (up to 10^{-4} *M*) also caused a marked transient increase in e.p.p. amplitude, but the effect developed much faster and complete blockade could be observed within 30 min. Figure 6 shows amplitude distributions of m.e.p.p.'s and e.p.p.'s before and after exposure to ATD. After about 1 hr of perfusion with 2.5×10^{-5} *M* ATD in Ringer's solution with low Ca^{2+}–high Mg^{2+} ratio the m.e.p.p. frequency was increased from 0.6–4.0 per sec. In this particular end-plate the time course of the ATD-effect was thus very slow. At this time the mean m.e.p.p. amplitude was only slightly decreased (from 0.39–0.34 mV), while the mean e.p.p. amplitude showed a marked increase (from 1.69–6.41 mV). In this experiment the quantal content (*m*) increased from a control value of 4.40 to a peak value of 22.98 [corrected for non-

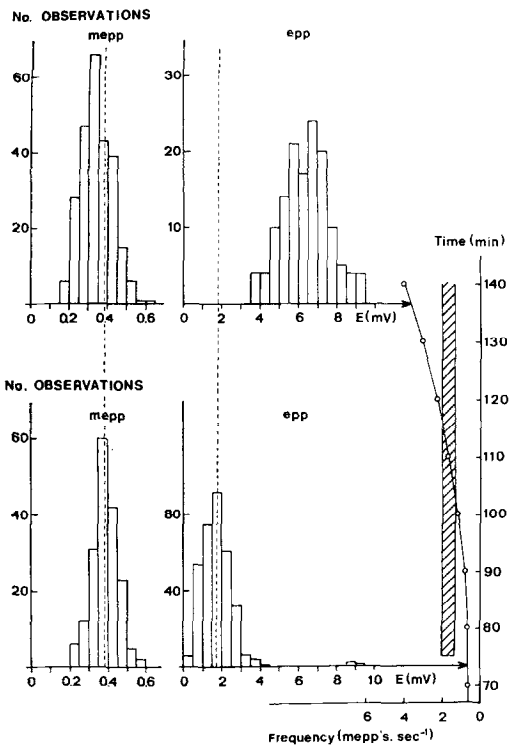

FIG. 6. *Effect of ATD on m.e.p.p. frequency and amplitude and on e.p.p. amplitude. M.e.p.p. and e.p.p. amplitude histograms were made before and at 62 min after addition of 2.5 × 10⁻⁵ M ATD as indicated by arrows. The dashed lines indicate the mean value of m.e.p.p. and e.p.p. amplitude before ATD addition. Time in minutes after impalement.*

linear summation, see ref. (23)]. In 12 other experiments with 1.0×10^{-5} or 2.5×10^{-5} *M* ATD the quantal content also showed a transient increase and *m* reached peak values of 2.5–5.9 times control value. The time of ATD-treatment to cause this increase in *m* varied between 7 min and more than 1 hr and was not clearly related to the ATD-concentration. After reaching its peak value, *m* gradually declined to values equal to or below control value. This is illustrated in Fig. 7 which shows the relation between m.e.p.p. and e.p.p. amplitude and quantal content *m* in a typical experiment. It was not possible to follow *m* further in time because the m.e.p.p.'s disappeared in the base line noise as described above. Provided the exposure to ATD was not too long, the effects of ATD were partially reversible and during washing with normal Ringer's solution there was usually a clear correlation between the increase in m.e.p.p. and e.p.p. amplitude and the fall in m.e.p.p. frequency.

In separate control experiments no effect of 0.1% ethanol on m.e.p.p. and e.p.p. amplitude was found. This is in agreement with Okada's observations (26). In most of our ATD-experiments a concentration of only 0.05% ethanol was used. Moreover, Okada (26) found an increase in m.e.p.p. amplitude after ethanol, while in our experiments ATD always caused a fall in m.e.p.p. amplitude.

Effects on ACh-potentials. Figure 8 shows the effect of ATD on the postjunctional membrane sensitivity towards ACh as estimated by ionophoretic application of ACh. The ACh-potential was gradually suppressed by 2.5×10^{-5} *M* ATD and 10 min after treatment with the drug had started its value was 61% of the control value. In the end the ACh-potential was

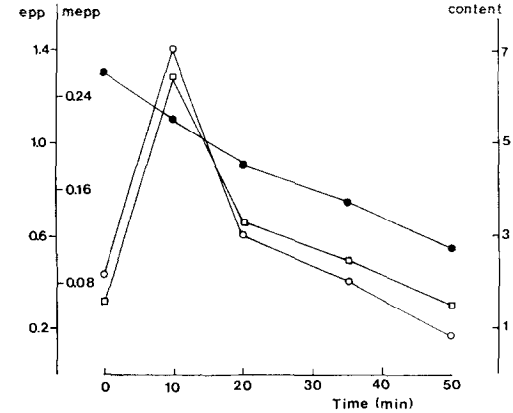

FIG. 7. *Effect of ATD on m.e.p.p. amplitude* (●), *e.p.p. amplitude* (○) *and on quantal content* (□). *Each point represents the mean value of 350 m.e.p.p.'s and 256 e.p.p.'s respectively. 2.5 × 10⁻⁵ M ATD is added to the bath at time 0 and is present throughout the experiment. Since the m.e.p.p. amplitude was suppressed by ATD, the lowest value of their amplitude was estimated to be equal to the modus of the amplitude histogram.*

almost completely blocked; after 35 min of ATD-exposure it was only 4% of the control value. In control experiments the ACh-potential remained essentially constant. In one preparation the ACh-potential was measured over a period of 40 min and did not show any significant change in amplitude (16.7 ± 1.2 mV; mean ±SD, $n = 21$). In 3 experiments the effect of ATD on ACh-potentials could partly be reversed by washing with normal Ringer's solution. In one experiment the suppression of the ACh-potential ran almost parallel to the fall in m.e.p.p. amplitude, while in three other experiments the ACh-potentials decreased faster in amplitude than the m.e.p.p.'s. The time course of the ACh-potentials was not greatly affected by ATD as can be seen in Fig. 8. These results clearly demonstrate that ATD suppresses the sensitivity of the end-plate membrane towards ACh.

Aldrin-Derived Dicarboxylic Acid (ADA)

Six nerve–muscle preparations were exposed for 90 min to ADA in a concentration of 5×10^{-5} *M*. No alteration either of m.e.p.p. frequency or m.e.p.p. amplitude was observed in any of these. Nor was the end-plate membrane potential affected. Afterwards, while the microelectrode was still in the same end-plate, the bathing solution was switched to Ringer's solution containing 2.5×10^{-5} *M* ATD. In all cases the m.e.p.p. frequency began to increase within a few minutes, showing the nerve terminal to be sensitive to ATD, but not to ADA.

9-Syn-hydroxy-HEOD (9HH)

Because of the small amount available, only 3 preparations were treated with this metabolite in a concentration of 2.5×10^{-5} *M*. No effect on m.e.p.p. frequency, m.e.p.p. amplitude and end-plate membrane potential was observed. After exposure to 9HH for 90 min these preparations responded well to 2.5×10^{-5} *M* ATD in the way described in the previous paragraph.

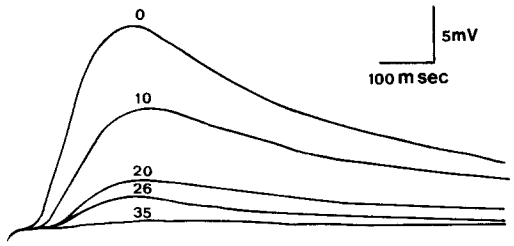

FIG. 8. *Depolarization of the end-plate membrane by ionophoretic application of ACh before* (○) *and at intervals after application of* 2.5×10^{-5} M *ATD. Numbers indicate time in minutes.*

DISCUSSION

The results of our experiments demonstrate that the HEOD metabolite ATD has a profound effect on synaptic transmission in the frog motor end-plate, exerting both pre- and postsynaptic actions. In contrast, HEOD itself and both other metabolites tested, ADA and 9HH, did not show any significant effect in the present study.

The presynaptic effects are difficult to bring into line with one another. First of all, ATD caused a rapid and often a more than fifty-fold increase in m.e.p.p. frequency. This increase in m.e.p.p. frequency is a highly reliable indicator for a presynaptic action of ATD. The most likely explanation for this effect is that ATD causes a depolarization of the nerve terminal. However, the ATD-induced increase in spontaneous transmitter release also occurred in Ca^{2+}-free Ringer's solution. Only when Ca^{2+} was withdrawn after the application of ATD or during the washout, a drop in the ATD-induced m.e.p.p. rate was observed. Since it is well known that the depolarization-coupled transmitter release requires the presence of Ca^{2+} in the external solution (27, 28), it appears that only part of the increase in m.e.p.p. frequency can be accounted for by a depolarization of the nerve terminal. The main effect of ATD is independent of the

presence of Ca^{2+} in the external solution. Such a Ca^{2+}-independent increase in spontaneous transmitter release, which is not yet understood, is also caused by black widow spider venom (30), high concentrations of ethanol (31) and several other drugs (32).

A second presynaptic effect of ATD is the inhibition of the increase in m.e.p.p. frequency induced by high external K^+. Not only could the K^+-induced m.e p.p. frequency not be sustained and declined to a lower level after treatment with ATD, but the maximum m.e.p.p. frequency was also greatly suppressed. An inhibition of the K^+-response has also been observed with neomycine, which probably competes or interacts with Ca^{2+} at some step in the prejunctional process leading to transmitter release (33), with Mn^{2+}, which suppresses the Ca^{2+}-permeability of the presynaptic membrane (34), and with tetanus toxin, which depletes transmitter stores (35). ATD may also have one of these effects, or ATD may have a Ca^{2+}-binding effect by itself. Another possibility is that ATD directly interferes with the mobilization of transmitter. Elmqvist and Feldman (36) observed a similar decline in K^+-induced m.e.p.p. rate, as described here, in untreated preparations which were exposed to 50 mM K^+. These authors suggested that this was due to a depletion of preexisting pools of ACh. After adding ATD this effect occurs with lower K^+-concentrations indicating that ATD inhibits the mobilization of ACh. Although the K^+-antagonistic effect of ATD lasted much longer than the increase in m.e.p.p. frequency, the maximum of both effects always coincided. This indicates that there may be a relation between the two effects.

ATD also causes a gradual fall of the m.e.p.p. amplitude and in most preparations all m.e.p.p.'s disappeared in the base line noise. The e.p.p. amplitude, on the other hand, began to show a marked increase after the application of ATD, which was more than threefold. This latter effect might be caused by an inhibition of ACh-esterase. This seems quite improbable, however, because the time course of decay of the e.p.p.'s and of the ACh-potentials was not affected by ATD. Nor was there an increase in m.e.p.p. amplitude or in the amplitude of the ACh-potentials. Besides, the increase in e.p.p. amplitude was accompanied by a marked increase in quantal content m. It would appear, therefore, that ATD enhances the process linking presynaptic depolarization to secretion of transmitter. Later on, e.p.p. amplitude and quantal content fell and in the end synaptic transmission was completely blocked. This might be caused by a depletion of the amount of releasable ACh, as argued above, or by a suppression of the nerve action potential. As far as this latter effect is concerned it is worth noting that along with the suppression of the e.p.p. an increase in delay between stimulus artifact and start of the e.p.p. was observed. This may suggest that ATD prolongs the synaptic delay (21), or that the nerve action potential is depressed. Preliminary experiments with frog sciatic nerve showed that exposure to ATD in a concentration of 5×10^{-5} M for 3 hr did not cause a significant reduction in the amplitude of the compound action potential.

Finally, it was shown that the sensitivity of the end-plate membrane to ACh is markedly inhibited by ATD. This postsynaptic blocking effect of ATD can account for most, if not all, of the fall in both m.e.p.p. and e.p.p. amplitude.

In summary, ATD has a rather complicated effect on frog motor end-plate causing presynaptically an increase in spontaneous transmitter release and a transient enhancement of evoked transmitter release, while postsynaptically it has a suppressive effect on ACh-sensitivity.

The ATD-effects on frog neuromuscular transmission described here are similar to the effects of dieldrin and ATD on synaptic transmission in the cockroach (1, 8–10). In both preparations there is

initially an enhancement of synaptic transmission, later followed by a blockade. According to Shankland and Schroeder (10) HEOD, or one of its metabolites, acts only presynaptically and the blockade would be the result of a complete depletion of transmitter. In the present study, however, a marked postsynaptic effect of ATD was demonstrated. The same authors suggest that in the cockroach the effect of HEOD, or a metabolite, is confined to cholinergic junctions. Whether the same applies to the frog cannot be concluded from our work. The action of ATD on the motor end-plate is probably not relevant to the symptoms of poisoning in the intact animal. It is conceivable, however, that ATD should have similar effects on synapses in the brain and the spinal cord. It is worth mentioning here that several drugs which cause central excitation have a distinct action on neuromuscular transmission (37).

Our findings strongly support the hypothesis of Wang *et al.* (8) that dieldrin must be converted to ATD before it can exert its neurotoxic action. To establish beyond doubt that ATD is the active form of the insecticide dieldrin this metabolite will have to be found in sufficient concentration in the nervous tissue of dieldrin poisoned animals to account for its action, as pointed out already by Wang *et al.* (8). So far, Matthews *et al.* (38) were not able to detect ATD in C^{14}-dieldrin fed rats. Recently, Sellers and Guthrie (39) observed by means of EM autoradiography that H^3-dieldrin was mainly located in synaptic regions of the CNS of the housefly, but this method does not distinguish between dieldrin and its metabolites. If the accumulation of ATD is restricted to synaptic regions only, the total concentration might be too low to be detected in whole brain extracts.

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